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Investigation of microbubble composition on ultrasonic dispersion properties for biosensing applications

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Abstract

Lipid shelled microbubbles are gaining attention as possible biosensors for monitoring the microbubble's in-vivo environment. These novel applications require the microbubble shell and gas components to be readily responsive to environmental changes. Since the ultrasonic properties of the microbubbles, for instance the resonance frequency or attenuation, are inherently related to the material properties of the monolayer shell such as visco-elasticity and thickness as well as on the physical properties of the encapsulated gas, it is important to investigate the influence of the shell composition and gas content on the ultrasonic behavior as well as the change in response after modifications of the microbubble environment. In this study, homemade microbubbles are characterized using ultrasonic through-transmission measurements in the range of 125 kHz to 10 MHz, thereby providing the dispersion relations of phase velocity and attenuation. Using this approach, the evolution of the dispersion properties of such bubbly media in time has been followed in order to detect changes in microbubble stability. In addition, several microbubble populations have been subjected to thermal changes to investigate their temperature dependence. The experimental observations have been compared to results from a nonlinear least squares fitting procedure with a theoretical model accounting for linear as well as nonlinear bubble behaviour. As such, the model allows to give a semi-quantitative interpretation of the dynamic behaviour and evolution of a microbubble population in the medium.

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Keywords: microbubble, contrast agent, dispersion properties, resonance frequency, dissolution, stability

1. Introduction

Encapsulated microbubbles also known as ultrasound contrast agents (UCA's), are widely used in diagnostic imaging [1]. UCA's improve the quality of ultrasound images by enhancing the signal in backscatter mode as well as in Doppler mode [2]. The functionality of microbubbles is expanding towards therapeutic applications such as gene transfer and drug delivery [3] and bio-sensing applications [4]. A thorough understanding and knowledge of the stability and acoustic signature of the microbubbles is of major importance in view of their effectiveness in any of their functionalities (as contrast agents, drug/gene-delivery vehicles or biosensors).

The acoustic properties are governed by the physical properties of the shell, the gas core and the local environment of the bubbles [5, 6]. In in-vivo situations microbubbles are subject to dissolution and phagocytosis causing rapid

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clearance in the vasculature [7]. Dissolution is driven by the Laplace pressure [8] and is enhanced in the presence of an ultrasound pressure field [9]. Consequently the acoustic properties of the microbubble population continuously change since these are strongly related to the microbubble size. In view of quantifying external parameters from the microbubble acoustic response, it is crucial to understand and quantify the variation of the acoustic signal itself. In this study, the evolution of the microbubble size and the dispersion properties of the medium are investigated in ultrasound through transmission experiments. The dispersion properties were examined at 23°C and 39°C to study the temperature dependence.

2. Materials and methods

2.1. Microbubble preparation

Air-filled DSPC:PEG40S (9:1 molar fraction) microbubbles were prepared using the lyophilization method as described by Schneider [10]. Briefly, DSPC (1, 2-distearoyl-*sn*-glycero-3-phosphatidylcholine, Avanti Polar Lipids, Alabaster, AL), PEG40S (polyoxyethylene-40 stearate, Sigma-Alrich) and PEG2000 (polyethylene glycol 2000, Sigma-Aldrich) are dissolved in tert-butanol (Sigma-Aldrich) and the mixture is freeze-dried for 1-2 days. The resulting powder is weighed and stored at 6°C in gas-tight brown glass storage vials with air filling the headspace. This freeze dried powder can be stored for weeks without a significant decrease in microbubble yield. Air-filled microbubbles are formed by dissolving the powder in Isoton II diluent (Beckman Coulter).

2.2. Optical microscopy

Bright field microscopy images were acquired with a Carl Zeiss Primo Star microscope (Zeiss, Germany). A microbubble solution of 2 μL is taken directly from the storage vial using a analytical syringe (SGE eVOL Analytical Syringe) and diluted with 10 μL air saturated Isoton II on a microscopy slide. Images are acquired every 30 seconds for 20 minutes and analyzed using ImageJ 1.47v (`http://imagej.nih.gov/ij`).

2.3. Ultrasound transmission measurements

The attenuation parameters of microbubble solutions were measured in an ultrasound through transmission set-up. The sample liquid is contained in a 2x2x4 cm glass holder. The glass holder features two circular openings on opposite sides to position two immersion transducers. An emitting and receiving transducer are separated by a distance of 2 cm and are in contact with the sample liquid. The liquid is continuously stirred. The temperature is controlled and monitored by a hot plate and an immersible temperature sensor (IKA, RCT basic and ETS-D5). All measurements were carried out with 10MHz transducers (Olympus, V311) for emission and reception. To investigate the dispersion characteristics, consecutive sinusoidal bursts were emitted with frequencies ranging from 0.125 to 10 MHz, with 125 kHz steps. At each frequency, the burst signal generated pressure levels below 62 kPa.

3. Results and Discussion

The dissolution behaviour of air-filled microbubbles in a stagnant medium was investigated by optical microscopy. Figure (1) (Left) shows the $R(t)$ curve of two air-filled microbubbles. The microbubble radius initially decreases and reaches an equilibrium value where dissolution stops. Katiyar *et al.* [8] has described this behaviour by incorporating the elasticity of the encapsulation in the Epstein-Plesset model [11]. In a saturated medium, a non-zero equilibrium radius can be reached when the interfacial tension γ_0 is counterbalanced by the encapsulation elasticity E^S giving $\gamma(R) = 0$ and correspondingly $dR/dt = 0$. As such, the model accounts for the high stability and long shelf-life of air-filled microbubbles. The size distribution after equilibration of the radii is given in figure (1) (Middle).

The dispersion of the attenuation of the microbubble medium obtained from the ultrasound transmission measurements is shown in figure (1) (Right). There is a narrow attenuation peak around 1.5 MHz with FWHM $\simeq$ 2 MHz.

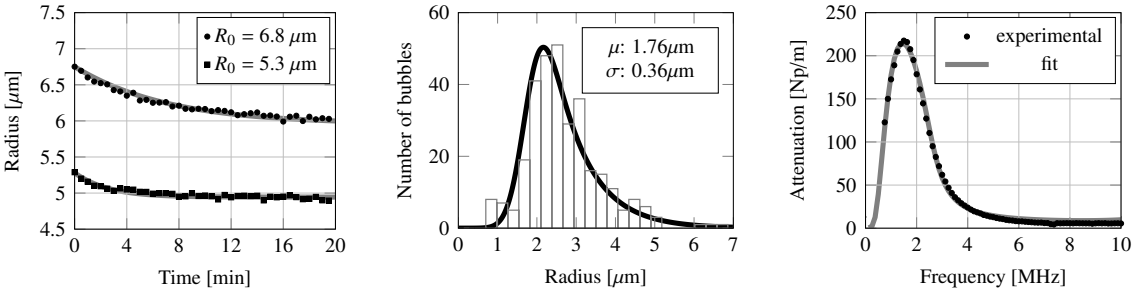

Fig. 1. (Left) Microbubble radius versus time obtained from bright field microscopy images. The behaviour is modeled using the dissolution model by Katiyar *et al.* [8]. (Middle) Size distribution of the microbubble population acquired after 20 minutes. The distribution is described by a skewed Gaussian function with center μ and standard deviation σ. (Right) The dispersion of the attenuation of the microbubble medium. Data were fit with a theoretical model accounting for shear thinning, strain-softening and strain-hardening behaviour [12].

The experimental data are fitted with a theoretical model accounting for shear thinning, strain-hardening and strain-softening behaviour of microbubbles [12, 13, 14], the parameters describing the size distribution were adopted from the optical microscopy analysis.

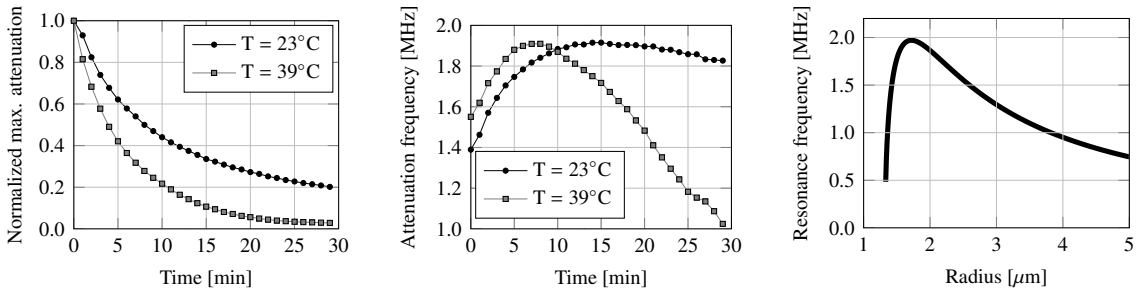

Fig. 2. (Left) Evolution of the maximal attenuation of the microbubble medium in time. (Middle) Evolution of the frequency at which maximal attenuation occurs. (Right) Resonance frequency as a function of radius adapted from the model by Marmottant *et al.* [15] and van der Meer *et al.* [16] with shell viscosity $\kappa_S = 1.6\ 10^{-8}$ kg/s and elasticity $\chi = 50$ mN/m.

The dispersion of the attenuation characteristics of the microbubble population is followed up in time. Figure (2) (Left) shows the evolution of the value of maximum attenuation. The attenuation decreases due to the continuous dissolution driven by the Laplace pressure and the ultrasound pressure field. The microbubbles continue to shrink, even when the equilibrium radius is reached. The faster decay in attenuation at 39°C emerges from the increased dissolution rate of the microbubbles caused by the increased diffusivity of the gas [17] and increased encapsulation permeability [18].

The frequency at which maximal attenuation occurs (see figure (2) (Middle)) initially increases, reaches a maximum value and subsequently decreases. We hypothesize this behaviour also results from the continuous decrease in microbubble size. In the model by Marmottant *et al.* [15], the resonance frequency f_{res} of lipid microbubbles in the elastic regime can be expressed as equation (1):

$$f_{res} = f_0\sqrt{1-\delta^2/2}, \text{ with } f_0 = \frac{1}{2\pi}\sqrt{\frac{1}{\rho R^2}\Big[3\kappa P_0 + \frac{2(3\kappa-1)\gamma_0}{R} + \frac{4\chi}{R}\Big]}, \text{ and } \delta = \frac{\omega_0 R}{c} + \frac{4\mu}{R^2\rho\omega_0} + \frac{4\kappa_S}{R^3\rho\omega_0} \qquad (1)$$

where $P_0 = 101.3$ kPa, $c = 1500$ m/s, $\rho = 1000$ kg/m^3, $\kappa = 1.4$, $\gamma_0 = 0.073$ N/m and χ, are the hydrostatic pressure, the speed of sound in the liquid, the density of the liquid, the polytropic exponent of air, the interfacial surface tension and the shell elasticity parameter in N/m, respectively. The resonance frequency is lower than the eigenfrequency f_0 due to damping δ which mainly arises from the shell dilatational viscosity κ_S, but also from the liquid viscosity μ, the

radiation damping and the thermal damping. For simplicity thermal damping is implemented as an effective viscosity taking $\mu = 2 \cdot 10^{-3}$ Pa s, as in van der Meer *et al.* [16].

Figure (2) (Right) shows the resonance frequency as a function of microbubble radius R. As the radius decreases from, for instance, 2.3 μm to 1.3 μm, the resonance frequency increases up to approximately 2 MHz and subsequently decreases as observed in the attenuation data. This trend is observed at 23°C as well as at 39°C where the dissolution rate is higher.

4. Conclusions

The long shelf-life and high stability of air-filled DSPC/PEG40-stearate microbubbles prepared via the lyophilisation method can be explained by the dissolution model of Katiyar *et al.* [8] which takes into account the shell elasticity. The dispersion properties of a bubbly medium were characterized by ultrasound transmission measurements. The maximal attenuation decreases in time as expected from the ultrasound-driven dissolution of the microbubbles. The decay rate increases with increasing temperature. The frequency at which the attenuation reaches its maximum initially increases as expected from the gradual decrease in mean radius of the microbubble population. The subsequent decrease in attenuation frequency follows from the contribution of the shell viscosity to the total damping coefficient.

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